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Effect of Chlorine on the Survival of *Vibrio cholerae* on Shrimp

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Shrimp is a an important commodity in international trade and stringent quality parameters stipulates zero tolerance of *Vibrio choleare* in the imported commodity. Sanitary requirements for production of good quality shrimp for processing and export stipulates that shrimp tissue is to decontaminated from pathogens like *Vibrio cholerae* by washing with potable water carrying chlorine .Hence effective dose of chlorine to be used in the process water is very critical. The present study was aimed at assessing the microbicidal efficacy of chlorine against *Vibrio cholerae* cells in water and shrimp with and without shell. The study indicated that in potable water with 2ppm residual chlorine level, a population of 10^7 cfu of *Vibrio cholerae* could be destroyed after 30 min exposure. Studies on shrimp meat contaminated with varying levels of *Vibrio cholerae* exposed to chlorine showed that 4ppm chlorine could effect complete destruction of a population of 10^3 cfu /ml in 10min. On headless shrimp with shell-on, 7ppm chlorine was required to destroy 10^3 cells /g of *V.cholerae* within 10 min. The data indicate that chlorine level has to be adjusted depending on nature of material and *Vibrio cholerae* present in shell-on shrimp need special attention.

Key words : *Vibrio cholerae* , shrimp, chlorine, survival

Toxigenic *Vibrio cholerae* O 1 and 139 are the causative agents of cholera, a water-borne and food borne disease with epidemic and pandemic potential. Various foods have been frequently implicated in the outbreak of cholera which includes milk and milk products, meat, vegetables, fruits and seafood (Tauxe *et al.*,1992). Outbreaks of cholera due to consumption of seafood, oyster, crab and shrimp have also been reported. (Oliver & Kaper ,1997).

V.cholerae may be considered as an indigenous member of estuarine / brackish water bacterial community with capability to survive long durations in adverse conditions (Singleton *et al.*,1982). It is more prevalent in aquatic systems during warm climates (Isalm *et al* 1992) but the pathogen can also survive in frozen shrimp for long duration (Wong *et al.*, 1995). *Vibrio cholerae* occur not only in coastal waters and shrimp from marine environments. (Depaola, 1981), but also in cultured fish (Fernandes *et al.*,1997), cultured freshwater shrimp, *Macrobrachium rosenbergii* (Jayasekaran & Ayyappan, 2002) and brackish water prawns (Reilly & Twiddy,1992). Fish and fishery products meant for export in India are found to carry both *Vibrio cholerae* O1 and non O1 (Varma

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The study was designed with following steps. To determine the effect of chlorine on *V. cholerae* suspended in potable water, one ml of *Vibrio cholerae* cell suspension of known cell densities were added to 100ml potable water containing varying levels of available chlorine in beaker under sterile condition. Immediately after the addition and at intervals of 5 min, 1ml aliquot was withdrawn and the excess chlorine was inactivated by adding to 10 ml of 2% Sodium thiosulphate. The viable count was determined immediately by spread plate method in tryptone glucose yeast extract agar TSYA (Oxoid) plates. Colonies of surviving bacteria were counted after incubation of the plates at 37°C for 24h. Trials were carried out separately for various cell densities (10^3 to 10^7 cells /ml) and chlorine levels (3-10 ppm).

In the case of shrimp, PD and HL, the experiment was repeated in the same way with following modifications. 1ml of *V.cholerae* cell suspension of varying cell densities as given above were added to 250 g each of HL and PD shrimp suspended in 1000ml chlorinated potable water in sterile condition as before. Shrimp (25kg) immersed in 100ml chlorine-free distilled water served as control. Immediately after the addition and at 5 min. intervals, 25 g lot of shrimp was taken out and the chlorine was neutralized by dipping in 100ml of 2% Sodium thiosulphate. Shrimp was then blended in 225 ml of saline, serially diluted and viable count was estimated by spread plating onto TSYA plates. Typical Colonies of surviving population were counted after incubation of the plates at 37°caviae for 24h. Since the background flora is destroyed by heat treatment, the survivors are considered to be *V cholerae* only. Occasionally about 5% of colonies from a plate were isolated and confirmed as *Vibrio cholerae* by standard protocol (Anon,2003)

Results and Discussion

Table 1 shows survival of *V.cholerae* when suspended in potable water with varying chlorine concentrations. At 2ppm residual level, no survivors could be noticed at any of cell densities after 30 min exposure. At 10 ppm level 5 min exposure was sufficient to completely destroy the peak level of 10^7 cells /ml. An earlier study on the effect of chlorine on *V.cholerae* has recommended chlorine level of 10 ppm and contact time of 5 min for total eradication of 1.80 x 10^6 cells ml. of *V. cholerae* in water (Iyer & Varma.,1991). Sousa et al (2001) reported that an initial density of 10^7cfu/ml. *V cholerae* in water suspension resulted in 100%reduction after 5 min exposure to 7 ppm chlorine.

Table 1 Viability of *V cholerae* in potable water after exposure to varying chlorine levels and exposure times

Time (Min.)	Cell density cfu/ml.	Survival(%) of *Vibrio cholerae* at different residual chlorine levels				
		2ppm	3ppm	4ppm	5ppm	10ppm
5	$1.68x10^7$	0.08	0.05	0.002	0.001	ND
10	$1.68x10^7$	0.001	0.01	0.001	ND	ND
20	$1.68x10^7$	0.001	0.001	ND	ND	ND
30	$1.68x10^7$	ND	ND	ND	ND	ND

ND - Not Detected

Table 2. Survival of *V cholerae* on shrimp (PD) after various exposure times and chlorine levels

chlorine ppm	Number of cells Exposedto Chlorine/ml.	Survival Rate % Range		Survival Rate % Mean*	
		5 min.	10 min.	5 min.	10 min.
2	$1.28x10^3$	2.88-3.38	1.36-1.80	2.98	1.52
3	$1.28x10^3$	2.11-2.40	1.48-1.77	2.29	1.62
4	$1.28x10^5$	0.42 – 0.98	0	0.62	0
5	$1.28x10^3$	0 – 0.20	0	.13	0
6	$1.28x10^3$	0	0	0	0
4	$1.28x10^6$	.11-0.17	0.2-0.4	0.14	0.03

* Mean of 3 experiments

The survival of *V. cholerae* associated with shrimp meat without shell is presented in Table2. When shrimp meat contaminated to a level of 10^3 to 10^6 cells /ml were exposed to chlorine, 4ppm chlorine could cause complete reduction of a population of 10^3 cfu /ml in

10min.When 10^6 cfu/ml were treated with 4ppm chlorine for 10 mints survival rate was 0.03%. This indicates that potable water for washing the shrimp meat carrying 10^6 cfu/ml *V cholerae* should carry 4 ppm of residual chlorine with contact time 10 min. Thus survival rate varies with number of cells exposed and the activity of chlorine.

Table 3. Survival of *V cholerae* on shrimp (HL) after various exposure times and chlorine levels

chlorine ppm	Cell density cfu/ml.	Survival Rate %Range 5 min.	Survival Rate %Range 10 min.	Survival Rate % Mean* 5 min.	Survival Rate % Mean* 10 min.
2	$1.28x10^3$	100	42.44-49.50	100	46.22
3	$1.28x10^5$	100	22.37-36.62	100	28.40
4	$1.21x10^3$	22.1-27.2	8.06-9.11	24.20	8.70
5	$1.28x10^5$	19.40-23.50	5.33-6.12	21.70	5.70
6	$1.21x10^3$	6.80-18.20	1.63-1.83	17.40	1.70
7	$1.28x10^5$	1.40-2.00	0	1.70	0
8	$1.28x10^3$	1.60-1.90	0	1.70	0
9	$1.28x10^3$	0	0	0	0
7	$1.28x10^5$	8.10-8.30	3.62-4.12	8.23	3.89

* Mean of 3 experiments

Table 3 shows the efficiency of chlorine on *V cholerae* present on headless shrimp with shell on. As evident from the data, 7ppm chlorine completely destroy 10^3 cells /g of *V.cholerae* within 10 mints. The chlorine level to bring out a similar effect on shrimp meat was only 4ppm. This clearly indicate that a higher chlorine concentration is required to treat shrimp with shell. At low chlorine levels of 2 and 3 ppm, the survival pattern for shrimp with and without shell was almost similar both in PD and HL shrimp and had very little bactericidal effect. After 4ppm chlorine ,there was striking decline in cell populations and more than one log reduction from the initial count was noted

Sousa (2001) reported a very slow decrease in *V cholerae* cell population with increase in chlorine concentration and attributed this to the capacity of bacterium to adhere tightly to chitin exoskeleton and escape from chlorine. Presence of excess organic matter(Alcamo,1994) protection afforded by chitin exoskeleton (Liu and Xie., 1994) , the decomposition products from chitin (Amako *et al.*, 1987) and greater adherence to shell (Pruzzo *et al.*, 1996) have been suggested as reason for the prolonged survival of *V cholerae* on shrimp with shell-on. The physical protection offered by hollow spaces in the carapace (Nascimento *et al.*, 1998) can also be an added reason. Apart from these, activity of any disinfectant may be greatly affected by the factors such as dilution, temperature, pH or the presence of organic matter . Hence the combination of all these factors could be responsible for the survival of *V cholerae* on whole shrimp.

In normal conditions , we do not expect any *V cholerae* cells in raw shrimp. Assuming that even in extreme situations, the maximum *V cholerae* count on shrimp may be 100 to 1000 cfu / g , the chlorine levels recommended in this study can ensure protection against survival or growth of *V cholerae*.. The study clearly shows that 2ppm chlorination levels used at present is insufficient to completely eradicate viable *V cholerae* present on shrimp although it may successfully lessen the external bacterial contamination. If safety of the consumer from the pathogen is the prime concern of the producer or buyer, disinfecting shrimp with appropriate dosage has to be strictly followed. By using low chlorine level, the surface contamination can be reduced, but pathogens may persist and pose health risk in later stages.

Development of chlorine resistant form of *V cholerae* has been reported previously (Morris *et al.*, 1996; Rice *et al.*, 1993) and such forms were noticed during this study also. Longer contact time of *V cholerae* with carapace

results in greater resistance and development of rugose forms (Sousa *et al.*, 2001). These chlorine resistant forms can also be a problem in future years if adequate chlorine levels are not maintained.

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